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MICROBIAL AND NUTRITIONAL EVALUATION OF BISCUITS PRODUCED FROM WHEAT AND QUALITY PROTEIN MAIZE FLOUR

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ABSTRACT

This study utilized composite flour from quality protein maize (QPM) and wheat for the production of biscuit with the bid to increase the protein quality of biscuit and promote the utilization of quality protein maize. Different blends of flour from quality protein maize and wheat with appropriate quantity of other ingredients were mixed to produce biscuits. The flour and biscuits produced were subjected to microbial, sensory and nutritional evaluations. The total viable counts of the samples ranged from $0.2x10^4$ CFU/g to 2.8 $x10^4$ CFU/g. Also, the total enterobacteriaceae counts ranged from 0.2 $x10^4$ CFU/g to 5.3 $x10^4$ CFU/g. Bacterial pathogens were isolated from the samples and the isolates were characterized and identified to be *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Bacillus subtilis* and *Proteus vulgaris*. *Bacillus subtilis* was found to be the most occurring bacteria species in both the flour and the biscuit samples. The nutritional evaluation of the biscuit samples showed that the 70:30 blend (wheat and quality protein maize) biscuit had the highest protein content (14.79%) while the 100% quality protein maize biscuit had the lowest (8.13%). The calorie value of the samples ranged between 2005.82 and 2235.206.05 KJ/mol. Wheat flour could be fortified with quality protein maize flour (at different ratio) to produce acceptable biscuits with improved protein composition i.e increased level of amino acids lysine and tryptophan at reduced cost.

KEYWORDS: Wheat, Quality protein maize, biscuit, blends, fibre contents and crude lipid.

INTRODUCTION

Snacks constitute the major menu in many people's diet day in day out and this may be due to tight schedule, nature of work, or natural likeness for snacks. Snacks like meat pies, sausage rolls, doughnuts, fish rolls, scotch eggs, cookies and biscuits are all abound in developed cities. Of all this snacks, biscuits constitute the major component of both young and old snacks in most part of the world. Biscuit is an unleavened crisp, sweet pastry snack made from wheat flour, at times contain composite of flours, hydrogenated fat and sugar, and is usually made light by the addition of a mixture of sodium carbonate, sodium bi- phosphate and cereal flour (O'Brien *et al.*, 2003).

The most common main ingredient for biscuit production is wheat flour coupled with margarine, yeast, eggs, milk, salt, flavours etc. Wheat (*Triticum aestivum*) is a highly nutritious plant; in 100 grams of wheat, 327 calories is produced and also rich in protein (13%), the 13% protein contains gluten which makes 75-80% of the protein in the wheat. High in carbohydrates (71%) and low in fat (1.5 %) (Shewry *et al.*, 2002). But about two or less decades ago, the use of composite flour has evolved. The extent at which wheat flour can be replaced with composite flour depends on the nature of the product to be baked. Composite flour has the added advantage of improving the nutrient value of biscuits and other bakery products, it was suggested that the ratio of 50:50 (wheat and non-wheat) flour mixture will be good for biscuit and other bakery products (Osuntogun, 1987). Composite flour is different from the wheat flour familiar with bakers and millers. It can be defined as mixture of flours from tubers rich in starch (e.g cassava, yam, sweet potato) and or protein-rich flours (e.g soy, peanut) and or cereals (e.g maize, rice, millet, etc), with or without wheat flour (Seibel, 1999).The essence of using composite flours in the making of biscuit is to improve the nutritional, organoleptic properties and shelf life of the biscuits. Many researchers have reported the use of composite flours for the production of biscuits over the years. Composite of sorghum and wheat flour (Adebowale *et al.*, 2012), wheat and plantain (Oyeyinka *et al.*, 2014), soybean and cassava (Akubor and Ukwuru, 2003), wheat and sweet potato (Onabanjo and Ighere, 2014), wheat and quality protein maize (Giwa and

Ikujenlola, 2010), wheat and maize bran (Usman *et al.*, 2015), acha and tiger nut (Ayo *et al.*, 2018) have been extensively reported. Much emphasis is being laid on the nutritional properties of the composite flour but less to none has been reported on the microbial properties. This study focused on both the microbial and nutritional evaluation of biscuits produced from wheat and quality protein maize flour. Quality Protein Maize (QPM) is product of plant breeding (not genetically modified) and it contains very high amount of alpha amino acids e.g lysine and tryptophan (Palit *et al.*, 2003). Quality Protein Maize (QPM) flour coupled with wheat flour would give unprecedented nutritional benefits and it would be more functional if it is free of microbial contaminant. This study is therefore aimed at evaluating the microbial and nutritional value of biscuits produced from composite of wheat and quality protein maize of different blends.

MATERIALS AND METHODS

Collection of Samples
The Quality Protein Maize (dried one), wheat flour with other baking ingredients like baking powder, sugar, vanilla flavor, nutmeg, preservatives, milk and egg were purchased at Sabo market in Ogbomoso, Oyo State.

Processing of Quality Protein Maize Flour
The quality protein maize was processed into flour by using the following unit operations: cleaning, drying, dehulling, degerming, milling, sieving and packaging.

Culture Media
The different media used for the isolation and culturing includes nutrient agar which is a general purpose media (for total viable counts); a selective media (Manitol salt agar) for total staphylococcus counts; differential media (MacConkey agar and Salmonella shigella agar) for total enterobacteriacae and Salmonella shigella counts respectively; potato dextrose agar for fungal counts. These were prepared according to the manufacturers' specification and sterilized at a temperature of 121°c for 15 minutes.

Isolation of Micro-Organism
One gram of each of the samples (quality protein maize and wheat flour) was dissolved (differently) into 9ml of sterilized water and shaken thoroughly to homogenize. Serial dilution was carried and one milliliter was drawn from the third dilution which was then introduced into in to petri dishes containing appropriate sterilized agar medium. It was then incubated at 37ºC for 24 hours. This was done before production (on the quality protein maize and wheat flour) and after production (on the biscuit blends).

Culture Examination
The incubated plates were checked after 24 hours for visible colonies after which the colonies were counted and expressed as colony forming units per gram (CFU/g).

Sub-Cuturing
Fresh medium was prepared and sterilized at 121°C for 15minutes and allowed to cool and poured aseptically into sterile petri dishes. The isolates were sub sub-cultured on new plates by streaking in order to get distinct pure colonies. These were incubated at 37°C for 24hours. After 24hours, the plates were observed for pure colony.

Culture Preservation
A colony was then picked from the plates, inoculate into a sterile nutrient agar slant, these were then incubated at 37°C for 24hours. After wards, it was preserved in the refrigerator at 4°C.

Formulation of Blends
The quality protein maize and wheat flour blends were made at varied ratios to give five blends. The ratios were (wheat: quality protein maize) 100:0; 70:30; 50:50; 30:70 and 0:100. This was done using the measuring cup.

Production of Biscuit
The various blends formulated were mixed separately with the same quantity of other ingredients (sugar, baking powder, water, butter, nutmeg, egg, milk, vanilla flavor and salt). The butter was mixed with sugar until fluffy using a mixer. The other ingredients were then added, and water was added until the desired texture of the batter was obtained. The batter was kneaded on a rolling table to acquire the desired thickness. The batter was later cut to different shapes with the aid of biscuit cutter. It was baked in an oven at a temperature of 160^{O}C for 15 minutes checking it once in a while until the biscuits are done. They were then cooled and packaged.

Sensory Evaluation
The organoleptic properties of the biscuit including taste, appearance, texture, and general acceptability were assessed by a 10- panel member using five point hedonic scale with 1 representing the least score (dislike extremely) and 5 the highest score (like extremely) was used.

Proximate Analysis of the Sample
Determination of moisture content
This method is based on moisture evaporation. Here the aluminum dishes were washed dried in oven and in desiccator for cooling. The weight of each dish was taken. 5.0 g of the samples were weighed into a sterile aluminum dish; weight of the dish and weight of undried sample (in duplicate) were taken. This was transferred into an oven set at 80°C for 2 h and at 100°C for 3 h respectively. This was removed and cooled in desiccator. Then the weight was measured using a measuring scale balance. It was transferred back into the oven for another one hour and then reweighed. The process continued until a constant weight was obtained. The difference in weight between the initial weight and the constant weight gained represents the moisture content.

Calculation: The loss in weight multiplied by 100 over the original weight is percentage moisture content.

$$Moisture\ content\left(\frac{g}{100g}\right) = \frac{\text{loss in weight (W2−W3)}}{\text{(W2−W1)}} X\ 100$$

Where W1= initial weight of empty crucible,
W2= weight of crucible + food before drying,
W3= final weight of crucible + food after drying.
% Total solid (Dry matter) (%) = 100- moisture (%)

Determination of Ash content
The ash represents the inorganic component (minerals) of the sample after all moisture has been removed as well as the organic material. The method is a destructive approach based on the decomposition of all organic matter such that the mineral elements may be lost in the process. Twenty grams (20 g) of each of the samples were weighed into a clean dried and cooled platinum crucible. It was put into a furnace set at 550 °C and allowed to blast for 3 h. It was then brought out and allowed to cool in a desiccator and weighed again.

Calculation: Percentage weight is calculated as weight of ash multiplied by 100 over original weight of the samples used.

$$Ash\ content = \frac{\text{Weight of ash}}{\text{Weight of original sample used}} X\ 100$$

Protein determination
Total protein was determined by the kjeldahl method as modified by Williams (1964). The analysis of a compound of its protein content by kjeldahl method is based upon the determination of the amount of reduced nitrogen present. About 20g of the samples were weighed into a filter paper and put into a kjedahl flask, 10 tablets of Na_2SO_4 were added with 1g of CuSO4 respectively. Twenty milliliter (20 ml) of conc. H_2SO_4 was added and then digested in a fume cupboard until the solution became colourless. It was cooled overnight and transferred into a 500 ml flat bottom flask with 200 ml of water. This was then cooled with the aid of packs of ice block. About 60 to 70 ml of 40% of NaOH was poured into the conical flask which was used as the receiver with 50ml of 4% boric acid using 3 days of screened methyl red indicator. The ammonia gas was then distilled into the receiver until the whole gas evaporated. Titration was done in the receiver with 0.01M HCl until the solution became colourless.

Calculation: The percentage protein is calculated as follows:

$$\%\,protein = Vs - Vb\ X\ 0.01401\ X\ N\ acid\ (6.25)\ X\ 100\ (original\ weight\ of\ sample\ used)$$

Where Vs = Volume (ml) of acid required to titrate sample,
Vb = Volume (ml) of acid required to titrate blank,
N acid = normality of acid.

Determination of lipid content
The method employed was the soxhlet extraction technique described by Shir law, (1967). 15 g of the samples were weighed and carefully placed inside a fat free thimble. This was covered with cotton wool to avoid the loss of sample. Loaded thimble was put in the Soxhlet extractor, about 200 ml of petroleum ether was poured into a weighed fat free soxhlet flask and the flask was attached to the extractor. The flask was placed on a heating mantle so the petroleum ether in the flask refluxed. Cooling was achieved by a running tap connected to the extractor for at least 6hrs after which the solvent was completely siphoned into the flask. Rotary vacuum evaporator was used to evaporate the solvent leaving behind the extracted lipids in the soxhlet. The flask was removed from the evaporator and dried to a constant weight in the oven at 60°C. The flask was then cooled in a desiccator and weighed. Each determination was done in triplicate. The amount of fat extracted was calculated by difference.

$$Ether\ extracts\ (100g)\ dry\ matter = \frac{\text{weight of extracted lipids}}{\text{weight of dry sample}} X\ 100$$

Crude Fibre Determination
The bulk of roughages in food is referred to as fiber and is estimated as crude fiber. Twenty grams (20 g) of the different samples were defatted with diethyl ether for 8 h and boiled under reflux for exactly 30 min with 200ml of 1.25% H2SO4. It was then filtered through cheese cloth on a flutter funnel. This was later washed with boiling water to completely remove the acid. The residue was then boiled in a round bottomed flask with 200ml of 1.25% sodium hydroxide (NaOH) for another 30 min and filtered through previously weighed couch crucible. The crucible was then dried with samples in an oven at 100°C, left to cool in a desiccator and later weighed. This was later incinerated in a muffle furnace at 600°C for 2 to 3 hours and later allowed to cool in a desiccator and weighed.

Calculation

$$\%\ fibre = \frac{\text{weight of fibre}}{\text{weight of original sample}} X\ 100$$

RESULTS AND DISCUSSION

Table 1 shows the microbial analysis of the quality protein maize and wheat flour before production. It indicates the total viable, staphylococcal, enterobacteriaceae and fungal counts of the flour samples. Total viable counts are used as a measure of microbiological quality with respect to the levels of the general microbial contamination. The total viable counts for quality protein maize and wheat flour were 2.2 x 10^4 and 2.6 x 10^4 CFU/g respectively with quality protein maize flour having the lowest total viable count. There was no enterobacteriaceae, salmonella/shigella and fungal counts for quality protein maize flour. The staphylococcal and enterobacteriaceae counts for wheat flour were 0.3 x 10^4 and 5.3 x 10^4 respectively.

Table 2 shows the total viable, enterobacteriaceae and fungal counts of the biscuits produced and that of the control. The total viable count of all the samples ranged from 0.2 x 10^4 to 2.8 x 10^4 CFU/g, the 70:30 (wheat: quality protein maize flour) biscuit had the lowest viable count (0.2 x 10^4) compared with the control (0.8 x 10^4). It was noted that 30:70 (wheat: quality protein maize flour) biscuits had the highest enterobacteriaceae count (2.5 x 10^4) as compared to 70:30 (wheat: quality protein maize flour) which had 0.2 x 10^4. Food production cannot be 100% free from microorganisms but can be controlled and minimized. Microbial evaluation of the samples was carried out and the microorganisms identified were *Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae, Bacillus subtilis, Proteus vulgaris*. Microorganisms have great importance and impact on our lives; they are fundamental for obtaining some food products like yogurt, cheese etc. However, they are also the main cause of food and cultivar deterioration. There are many factors that contribute to the presence of microorganisms in foods; the endogenous presence and cross contaminations being the most important (Adams and Moss, 1995). Microorganisms isolated often get into food through poor and non-hygienic handling of equipments and materials used in food production, for example, contaminations through wrapping and packaging (FSA, 2013). The distribution of the isolates in the flour samples and the biscuits produced from different wheat and quality protein maize flour blends is shown in Table 3. *Bacillus subtilis* was found to be the most occurring bacteria species in both the flour and the biscuit samples.

Organoleptic properties are the aspects of food, water or other substance that an individual experiences via the senses including taste, sight, smell and touch. The picture of the biscuit of different blends produced is shown in Figure 1 and the statistical analysis of the organoleptic properties of the biscuit samples are presented in Table 4. The sensory evaluation was to determine the general acceptability, texture, taste and appearance of the biscuit samples. Analysis of variance (ANOVA) was performed on the data obtained from the view of the panellists to determine differences, while the Duncan multiple range test was used to separate means where significant difference existed. The appearance and texture of the biscuit samples were all acceptable to the panellists and there was no significant difference ($P>0.05$). However, the 30:70 biscuits were most preferred in terms of appearance and texture. The taste and general acceptability shows that there was a significance difference ($P<0.05$). And, 100% wheat flour biscuits were most preferred in terms of taste and general acceptability which was also in agreement with the reports of Giwa and Ikujenlola (2010) that also used quality protein maize as composite flour.

Table 5 shows the proximate composition of the biscuit samples. It showed that 100% wheat flour biscuits had the highest moisture and ash contents of 6.44 and 5.02% respectively compared to others. The nutritional evaluation carried out in this study showed that there is reduction of moisture content upon fortification with quality protein maize flour. Reduction in moisture content reduced the chances of spoilage by microorganisms and consequently guarantees good storage stability; this is in agreement with the findings of Giwa and Ikujenlola (2010) and Ayo *et al* (2018). Also, according to Adebowale *et al.* (2012) baked foods such as cake, cookies and bread with high moisture content encourages bacterial, yeast and mould growth that could lead to spoilage. However the biscuits produced have low moisture for safe storage and inhibition of microbial growth that could affect their quality. The ash content of the biscuits produced was in the same range except for that of 100%wheat flour, this also goes in line with the work of Giwa and Ikujenlola (2010). It is the inorganic residue remaining after the removal of water and organic matter by heating in the presence of oxidizing agent (Sanni *et al*., 2008). It aids the metabolism of other compounds such as fat, protein and carbohydrate (Okaka and Ene, 2005).

The 70:30 wheat/quality protein maize flour biscuit had the highest protein content of 14.79% while the 100% quality protein maize biscuit had the lowest (8.13%). The 30:70 blend biscuit had the highest protein content which is in agreement with the report of Giwa and Ikujenlola (2010). This also conforms to the report of Omeire and Ohambele (2010) who reported increase in protein content in cookies produced from wheat-defatted cashew nut flour blends. Olaoye *et al* (2006) also observed an increase in the protein content with corresponding increase in the proportion of soy flour supplementation in cookies produced from composite flour of wheat, plantain and soybean.

The 100% quality protein maize biscuit had the highest crude fibre and calorific value of 2.43% and 2235.21KJ/mol. The presence of fibre in the food is essential owing to its ability to facilitate bowel movement (peristalsis), bulk addition to food and Prevention of many gastrointestinal diseases (Satinder *et al.,* 2010). The 30:70 wheat/quality protein maize flour biscuit had the highest carbohydrate and crude lipid contents of 78.76 and 18.11% respectively. The presence of high fat content in the biscuits serves as lubricating agent that improves the quality of the product in terms of flavor and texture. In addition, fat is a rich source of energy and is essential as carriers of fat soluble vitamins; A, D, E and K.

This study shows that wheat flour could be fortified with quality protein maize flour (at different ratio) to produce acceptable and good quality biscuits. The incorporation of the quality protein maize flour ensures that the biscuit have an improved protein composition i .e increased level of amino acids lysine and tryptophan and therefore of higher nutritive value at reduced cost. This will in turn increase the marketability of the biscuit and boost the economy of the biscuit industries and the nation at large.

Table 1: Microbial Evaluation of the Flours (in CFU/g) before Production.

Sample	TVC	SC	TEC	SSC	FC
QPMF	2.2 x10^{4}	0.6 x10^{4}	-	-	-
WF	2.6 x10^{4}	0.3 x10^{4}	5.3 x10^{4}	-	-

TVC (Total Viable Counts); SC (Staphylococcus Counts); TEC (Total Enterobacteriaceae Counts); SSC (Samonella Shigella Counts); FC (Fungal Counts); QPMF (Quality Protein Maize Flour); WF (Wheat: Flour).

Table 2: Microbial Evaluation of the Biscuits (in CFU/g) after Production.

Sample	TVC	FC	TEC
A	2.0 x 10^{4}	-	1.0 x 10^{4}
B	2.8 x 10^{4}	-	1.8 x 10^{4}
C	1.3 x 10^{4}	-	1.2 x 10^{4}
D	0.5 x 10^{4}	-	0.2 x 10^{4}
E	0.2 x 10^{4}	-	2.5 x 10^{4}
F	0.8 x 10^{4}	-	1.5 x 10^{4}

TVC (Total Viable Counts); TEC (Total Enterobacteriaceae Counts); FC (Fungal Counts); Control: Industrially Produced Biscuit A (100% Wheat flour biscuit); B (100% Quality protein maize flour biscuit); C (50% Wheat: 50% Quality protein maize flour biscuit); D (70% Wheat: 30% Quality protein maize flour biscuit); E (30% Wheat: 70% Quality protein maize flour biscuit); F (Control Biscuit).

Table 3: Distribution of the Isolates in the biscuit produced.

Samples	*Escherichia coli*	*Staphylococcus aureus*	*Klebsiella pneumoniae*	*Bacillus subtilis*	*Proteus vulgaris*
QPMF	+	+	-	+	-
WF	-	-	+	+	-
A	-	-	-	+	+
B	-	+	-	+	-
C	-	-	-	+	+
D	-	-	-	+	-
E	-	-	-	+	+
F	-	-	-	+	-

QPMF (Quality Protein Maize Flour); WF (Wheat Flour); A (100% Wheat flour biscuit); B (100% Quality protein maize flour biscuit); C (50% Wheat: 50% Quality protein maize flour biscuit); D (70% Wheat: 30% Quality protein maize flour biscuit); E (30% Wheat: 70% Quality protein maize flour biscuit); F (C).

Table 4: Sensory Evaluation of the Biscuits Produced.

Sensory parameters	A	B	C	D	E
Appearance	4.11^{a}	4.11^{a}	4.22^{b}	3.89^{a}	4.78^{c}
Taste	4.67^{b}	4.11^{a}	3.78^{a}	3.89^{a}	3.44^{c}
Texture	4.44^{b}	4.33^{a}	4.33^{a}	3.89^{a}	4.78^{c}
General Acceptability	4.44^{c}	4.22^{b}	4.22^{b}	3.89^{a}	3.00^{a}

Mean value with different subscript in the same row are significantly different (P<0.05).

A (100% Wheat flour biscuit); B (100% Quality protein maize flour biscuit); C (50% Wheat: 50% Quality protein maize flour biscuit); D (70% Wheat: 30% Quality protein maize flour biscuit); E (30% Wheat: 70% Quality protein maize flour biscuit).

Table 5: Nutritional Evaluation of the Produced Biscuit.

Samples	Moisture %	Ash %	Protein %	Crude Fibre %	Carbohydrate %	Crude lipids %	Calorific Value KJ/mol
A	6.44	5.02	14.50	2.35	71.98	15.02	2005.82
B	5.17	4.92	8.13	2.43	77.94	16.58	2235.206
C	3.57	4.95	12.29	2.35	75.15	13.60	2060.05
D	1.99	4.92	14.79	2.34	77.10	13.93	2058.17
E	3.57	4.92	10.50	2.26	78.76	18.11	2173.31

A (100% Wheat flour biscuit); B (100% Quality protein maize flour biscuit); C (50% Wheat: 50% Quality protein maize flour biscuit); D (70% Wheat: 30% Quality protein maize flour biscuit); E (30% Wheat: 70% Quality protein maize flour biscuit).

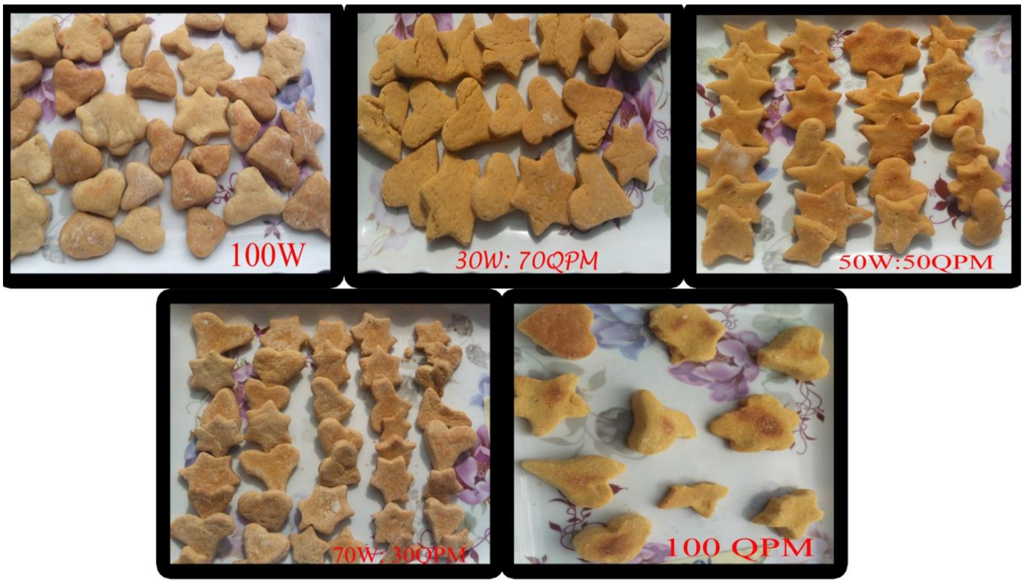

Figure 1: Biscuit of different blends produced.

100W (100% Wheat flour biscuit); 30W:70QPM (30% Wheat: 70% Quality protein maize flour biscuit); 50W:50QPM (50% Wheat: 50% Quality protein maize flour biscuit); 70W:30QPM (70% Wheat: 30% Quality protein maize flour biscuit); 100QPM (100% Quality protein maize flour biscuit).

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